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We proposed to test the validity of the hypothesis that introduction of recombinant toxins into the confines of the mammary ductal tree through the teat will kill breast epithelial cells. The toxins TGF-α and Heregulin-linked Pseudomonas exotoxin would be tested in the rat MNU-induced mammary tumor model. In the first year, we injected varying amounts of the TGF-a/PE and the Heregulin/PE toxin in rats by the intraductal route. While the toxin was extremely potent in human normal and breast cancer cells in culture, it was ineffective in killing the rat ductal cells. Although highly conserved, the human ligands do not appear to bind to the rodent receptors. We needed to design new toxins to target rat cells. We have completed the construction of a chimeric toxin consisting of the protein transduction domain of the HIV TAT gene to target and enter the cells, and the VPR gene of HIV to cause apoptosis. Expression of this protein in bacteria, and its purification is in progress. In the second year of this grant we will test the efficacy of this toxin in cultured human and rat breast cells. If effective, we will test the toxin in the rat model system.

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INTRODUCTION

Women with a strong family history of breast cancer may have up to an 80% risk of developing breast cancer over their lifetime. Given the rising risk, and the increasingly identifiable high risk group, the time has come to give serious consideration to the options available to prevent breast cancer. The matter has acquired a sense of urgency in the last three years because of two seminal discoveries in the genetics of hereditary breast cancer. Individuals with a family history of breast cancer (comprising 5-10% total breast cancer cases) often carry a mutation in the breast cancer susceptibility genes BRCA1, BRCA2 or p53 and ATM are at particularly high risk of developing breast cancer at a young age. As more women test positive for mutations in BRCA1 and BRCA2, the question of how best to manage these patients becomes ever more pressing. Unless reliable and effective methods for preventing breast cancer can be devised, determining susceptibility to breast cancer may be useless and possibly even psychologically detrimental. As more breast cancer associated genes are identified, particularly among the larger population of women without a strong family history, preventive strategies with minimal side effects are clearly needed.

BODY

This proposal seeks to test the radically new idea that breast cancer prevention can be achieved by selectively killing the cells that line the ducts from which the majority of malignant breast cancers arise. Multiple strategies could be applied to selectively kill breast epithelial cells. One method is to use proteins called ligand/toxin conjugates. Ligand/toxin conjugates combine their cytotoxic properties with the ability to selectively target cells carrying specific growth factor receptors. Cells that do not express the receptors remain unaffected.

1. Determine if mammary epithelial cells are susceptible to the cytotoxic activity of TGFα/PE38, HELβ1/PE38, or HELβ2/PE38 recombinant proteins in vivo.

We initiated experiments to determine the LD50 of the toxins when administered by the novel intraductal route in rats and the dose of toxin that will effectively ablate the mammary gland. Following injection of upto 2 ug of toxin per rat, no generalized toxic effects such as weight loss or death of mammary ducts as determined by microscopic examination of sections of the mammary gland was observed. We raised the dose upto 4 ug, with no effect. Neither of the two toxins was effective, suggesting that neither the EGF receptor or the neu receptor was binding to the cognate human ligand linked toxin.

In the face of these observations, it was clear that we needed to design a new ligand/toxin that would be lend itself to testing in rodent model systems and is then translatable to humans.

Delivery of cytotoxic proteins as a means of prevention or therapy is hampered by their size and biochemical properties. Work from Steve Dowdy's laboratory has demonstrated the efficiency of delivery of large proteins by fusing them to a 12-amino acid protein transduction domain of the HIV TAT protein. This protein transduction happens in a swift, concentration-dependent fashion is independent of receptors. Instead it targets the lipid bilayer of the membrane of the cell. So, in theory, all mammalian cell types should be susceptible to this mode of protein transduction. Further the same work goes on to show internalization and expression of the same TAT protein linked to -βGAL protein in various mouse organs in vivo. The purified proteins were denatured while the injected proteins were renatured intracellularly.

Another HIV gene VPR (Viral Protein) encodes a protein which induces G2 arrest and apotosis in a variety of cell types by a direct effect on the mitochodrial permeability transition pore. Amino acids 53 to 96 of VPR encompass a basic domain that is sufficient and responsible for this apoptotic effect.

So in the next year we will test the concept that administration of TAT-VPR fusion protein through the nipple can lead to a novel method of treatment as well as prevention of breast cancer caused by MNU. It will be first tested in cytotoxicity assays in cultured cells. Next the LD50 will be determined in rats. Finally, we will use the NMU-treated rat mammary tumor model system to test its efficacy in vivo. The protein will be delivered by intraductal administration to the rats. End points will be tumor incidence, latency of onset, size and overall survival rates at the end of 175 days.

KEY RESEACH ACCOMPLISHMENTS

- synthesized the TAT-VPR chimeric gene using oligosynthesis. Cloned the gene into an expression plasmid and expressed the protein using a bacterial E. Coli system.

REPORTABLE OUTCOMES

None to date

CONCLUSIONS

Detailed experimentation on the in vivo effects of TGFα-PE toxin and Heregulin/PE toxin revealed that the toxins are specific to human cells, and had no action on rat ductal cells. No toxic effects were discernible even at very high doses of 4 ug per rat. A novel toxin was designed using reagents that contain TAT domains that are internalized by all mammalian cells and the domain of VPR that causes apoptosis in cells. The chimeric protein will be injected alone and also as liposome preparations to achieve nonsurgical removal of preneoplasias as well as dividing cells in the breast.

REFERENCES

None

APPENDICES

None